Fludarabine, Treosulfan and Etoposide Sensitivity and the Outcome of Hematopoietic Stem Cell Transplantation in Childhood Acute Myeloid Leukemia

JAN STYCZYNSKI[1], JACEK TOPORSKI[2], MARIUSZ WYSOCKI[1], ROBERT DEBSKI[1], ALICJA CHYBICKA[2], DARIUSZ BORUCZKOWSKI[3], JACEK WACHOWIAK[3], BEATA WOJCIK[4], JERZY KOWALCZYK[4], LIDIA GIL[5], WALENTYNA BALWIERZ[6], MICHAL MATYSIAK[7], MARYNA KRAWCZUK-RYBAK[8], ANNA BALCERSKA[9] and DANUTA SONTA-JAKIMCZYK[10]

[1]Department of Pediatric Hematology and Oncology, Medical College, Nicolaus Copernicus University, ul. Curie-Sklodowskiej 9, 85-094 Bydgoszcz;
[2]Department of Pediatric Transplantology, Hematology and Oncology, Medical University, ul. Bujwida 44, 50-345 Wroclaw;
[3]Department of Pediatric Transplantology, Hematology and Oncology, Medical University, ul. Szpitalna 27/33, 60-572 Poznan;
[4]Department of Pediatric Hematology and Oncology, Medical University, ul. Chodzki 2, 20-093 Lublin;
[5]Department of Hematology, Medical University, ul. Szamarzewskiego 84, 60-569 Poznan;
[6]Department of Pediatric Oncology/Hematology, Medical College, Jagiellonian University, ul. Wielicka 265, 30-663 Krakow;
[7]Department of Pediatric Hematology and Oncology, Medical University, ul. Marszalkowska 24, 00-576 Warsaw;
[8]Department of Pediatric Hematology and Oncology, Medical University, ul. Waszyngtona 17, 15-274 Bialystok;
[9]Department of Pediatric Hematology, Oncology and Endocrinology, Medical University, ul. Debinki 7, 80-210 Gdansk;
[10]Department of Pediatric Hematology and Oncology, Medical University, ul. 3 Maja 13/15, 41-800 Zabrze, Poland

Abstract. *Background: The prognostic role of the ex vivo drug resistance profile has not yet been proved in childhood acute myeloid leukemia (AML). The aim of the study was the analysis of the impact of the ex vivo drug resistance profile in a cohort of 44 children with AML undergoing hematopoietic stem cell transplantation (HSCT). Patients and Methods: Myeloblasts for drug resistance testing were obtained from the bone marrow either on diagnosis or at relapse, before the HSCT procedure and were tested by the MTT assay. Results: Children who relapsed after transplantation showed higher ex vivo resistance of the leukemic blasts to etoposide, mercaptopurine, thioguanine, fludarabine, mitoxantrone and treosulfan than those who stayed in remission. Despite being nondiscriminative, the combined ex vivo drug resistance profile to fludarabine, treosulfan and etoposide (FTE score) was the strongest prognostic factor by multivariate analysis. Conclusion: The combined drug resistance profile to fludarabine, treosulfan and etoposide may be useful for better stratification of children with AML undergoing stem cell transplantation or to indicate the necessity for additional post-transplant therapy.*

Correspondence to: Jan Styczynski, MD, Ph.D., Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University, ul. Curie-Sklodowskiej 9, 85-094 Bydgoszcz, Poland. Tel: +48 601 222 131, Fax: +48 52 585 4867, e-mail: jstyczynski@cm.umk.pl

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The combined *ex vivo* drug resistance profile to prednisolone, vincristine and L-asparaginase (PVA score) has prognostic value in childhood *de novo* acute lymphoblastic leukemia (ALL) (1, 2). The results of therapy for childhood acute myeloid leukemia (AML) differ from those of ALL. The development of drug resistance is the limiting factor in the therapy of AML (3). In pediatric AML, in spite of several reports (3-5), the prognostic role of the *ex vivo* drug resistance profile has not yet been proved, with one possible exception of the prognostic role of sensitivity to cytarabine (6), while this relationship has been confirmed in adult AML (7). Apart from one study of children and adults with acute leukemia (8), no data are available regarding the possible role of drug resistance in children undergoing hematopoietic stem cell transplantation (HSCT). In this study, the prognostic value of the *ex vivo* drug resistance profile in pediatric AML patients undergoing HSCT was analyzed.

Patients and Methods

Patients. Fourty-four children (28 male, 16 female) with AML, including 20 in complete remission (CR1) and 24 relapsed patients, aged 0.7-17 years (median 10 years), who underwent HSCT, were included in the study. Patients with favourable cytogenetics of the leukemic blasts and those suffering early transplant-related mortality were not included in the study. The patients received myeloablative conditioning based on busulfan (n=32), treosulfan (n=8), fractionated total body irradiation (FTBI, n=3), or cyclophosphamide (n=1). Thymoglobulin was administered before unrelated or haploidentical HSCT. The source of the graft was matched sibling donor (n=20), matched unrelated donor (n=9), autologous (n=12) or mismatched family donor (n=3). The patients were followed up for a median of 2.5 (range 0.2-4.3) years. Fresh myeloblasts for drug resistance testing were obtained from the bone marrow either on diagnosis or at relapse, before HSCT, and were processed as described previously (9). Only samples with at least of 70% of myeloblasts were included in the study. The study was approved by the local Bioethical Committee and written informed consent was obtained from all patients and their parents.

Drug resistance profile. The drugs used in the study are listed in Table I. The cytotoxicity of the tested compounds to leukemic cells was measured by the MTT assay, as described previously (10). All experiments were performed in duplicate. The cytotoxicity was expressed as IC_{50}, the inhibitory concentration for 50% of the cells. According to the median cytotoxicity for each tested drug, all the patients were scored as resistant (score 2) or sensitive (score 1) to this drug. The FTE score was defined as the sum of three respective score values for fludarabine, treosulfan and etoposide; thus the FTE score ranged from 3 to 6. An FTE score of 3-4 was regarded as sensitive and 5-6 as resistant.

Statistical analysis. The Mann-Whitney *U*-test was used for unpaired comparisons. Survival curves were calculated by the Kaplan-Meier method and compared by the log-rank test. The Cox proportional hazards regression model was used in univariate analysis. The significantly important factors were fitted together in multivariate analysis in a backward stepwise manner using the likelihood ratio test until all factors in the model were significant. All reported *p*-values are two-sided, $p<0.05$ was considered as statistically significant.

Results

Relapses after HSCT occurred in 15/44 children. The children who relapsed after HSCT showed higher *ex vivo* resistance of their leukemic blasts to fludarabine, treosulfan, etoposide, mercaptopurine, thioguanine and mitoxantrone (Table I). No significant differences were found for the other drugs.

The overall probability of disease-free survival (pDFS) for all 44 patients was 0.64±0.07, with a mean survival time of 2.7 years [95% confidence interval (CI)=2.1-3.3]. The type of HSCT, disease status and age at transplantation had no influence on the pDFS (Figure 1A-C). Better pDFS was observed for patients *ex vivo* sensitive to fludarabine (0.80±0.10 *vs.* 0.41±0.12, $p=0.0184$), thioguanine (0.81±0.09 *vs.* 0.44±0.11, $p=0.0090$), treosulfan (0.85±0.09 *vs.* 0.46±0.13, $p=0.0368$), etoposide (0.89±0.07 *vs.* 0.47±0.10, $p=0.0038$), and for patients with a sensitive combined drug resistance profile (FTE score) (0.91±0.08 *vs.* 0.46±0.12, $p=0.0221$) (Figure 1D).

Cell-biological features (age, sex, blast morphology, initial leukocytosis, cytogenetics), response to therapy and drug resistance profile parameters were taken together in the Cox model. Out of all tested factors, those predicting positive outcome by Cox univariate analysis were: *ex vivo* sensitivity to fludarabine [$p=0.036$, hazard ratio (HR)=0.50, 95%CI=0.26-0.95], etoposide ($p=0.016$, HR=0.40, 95%CI=0.19-0.85), treosulfan ($p=0.018$, HR=0.46, 95%CI=0.24-0.87), thioguanine ($p=0.048$, HR=0.59, 95%CI=0.21-0.97), and a sensitive combined drug resistance profile discriminated by FTE score ($p=0.014$, HR=0.44, 95%CI=0.18-0.83). No factors showed prognostic value by multivariate analysis, however the sensitive combined drug resistance profile discriminated by FTE score was the strongest prognostic factor ($p=0.076$, HR=0.39, 95%CI=0.13-1.10), though it was possible to define the FTE score in only 33 out of 44 patients.

Discussion

The drug resistance profile identifies patients at higher risk of treatment failure. In the study of Miller *et al.*, which included children and adults with ALL and AML, the sensitivity of the occult leukemia colony-forming units to 4-hydroperoxycyclophosphamide was the only factor that predicted relapse following HSCT (8). In the present study, the combined *ex vivo* drug resistance profile, although being nondiscriminative, was the strongest prognostic factor in a multivariate analysis for AML children undergoing HSCT. No firm conclusions regarding the necessity for the use of fludarabine, treosulfan and etoposide in the treatment of AML with HSCT can be drawn from our study; however these results might indicate that patients whose myeloblasts are sensitive to fludarabine, treosulfan and etoposide would benefit from the use of these drugs during conditioning before HSCT, and possibly at the earlier stages of therapy. Resistance to these drugs might suggest the necessity of implementation of post-transplant procedures, such as close monitoring of minimal residual disease, reduction of immunosuppressive therapy, immunotherapy based on donor lymphocyte infusion, or administration of interleukin-2. The FTE score, however, could not prevent the overtreatment of the patients with sensitive FTE scores, as all patients qualifying for HSCT need very intensive therapy.

AML is a relatively rare disease in children, however, the results of therapy for this disease are still not satisfactory, as only 50%-60% remission has been reported (11-14). The value of testing sensitivity to fludarabine, treosulfan and

Table I. *Comparison of ex vivo drug resistance in patients staying in remission and relapsing after HSCT. The MTT assay was performed at AML diagnosis, before HSCT was planned.*

Drug (Company)	Concentration [μM]	IC_{50} (median and quartiles)		RR	*p*-value
		Remission	Relapse		
Cytarabine	0.04-41	2.38 (n=28)	2.71 (n=15)	1.13	0.838
(Pharmacia Upjohn, Bentley, Australia)		0.57-9.66	1.35-5.13		
Cladribine	0.001-140	0.26 (n=28)	0.24 (n=15)	0.94	0.508
(Bioton, Warsaw, Poland)		0.04-2.32	0.12-4.44		
Fludarabine phosphate	0.05-54	2.16 (n=20)	8.68 (n=13)	4.01	0.036*
(Schering AG, Berlin, Germany)		0.87-8.15	3.25-33.1		
Daunorubicin	0.002-3.5	1.01 (n=28)	1.25 (n=15)	1.24	0.177
(Rhone-Poulenc-Rhorer, Paris, France)		0.54-1.50	0.95-2.16		
Doxorubicin	0.01-13.8	4.61 (n=22)	11.2 (n=12)	2.32	0.720
(Farmitalia, Milan, Italy)		1.31-13.8	1.56-13.8		
Epirubicin	0.003-3.4	1.79 (n=20)	1.19 (n=11)	0.66	0.495
(Farmitalia, Milan, Italy)		0.93-3.13	0.75-2.51		
Idarubicin	0.003-3.7	0.82 (n=27)	0.80 (n=15)	0.97	0.609
(Zavedos, Pharmacia, Milan, Italia)		0.35-1.53	0.39-1.12		
Mitoxantrone	0.002-1.9	0.67 (n=18)	1.33 (n=11)	1.97	0.045*
(Jelfa, Jelenia Gora, Poland)		0.42-1.02	0.34-1.9		
Etoposide	0.08-85	23.6 (n=28)	47.6 (n=14)	2.01	0.018*
(Bristol–Myers Squibb, Princeton, NJ, USA)		4.12-43.2	34.7-85		
6-Thioguanine	9.3-299	45.9 (n=21)	130.9 (n=14)	2.84	0.006*
(Sigma, A4882, St. Louis, MO, USA)		25.6-103.8	79.2-299		
6-Mercaptopurine	91-2937	418 (n=19)	1328 (n=14)	3.17	0.012*
(Sigma, M7000, St. Louis, MO, USA)		206-1038	629-1981		
4-HOO-cyclophosphamide	0.3-341	6.85 (n=23)	10.6 (n=10)	1.55	0.153
(Asta Medica AG, Frankfurt/Main, Germany)		3.65-14.0	5.66-33.1		
4-HOO-ifosfamide	0.33-341	59.0 (n=9)	64.9 (n=6)	1.10	0.679
(Asta Medica AG, Frankfurt/Main, Germany)		21.4-130.8	14.9-116.4		
Mafosfamide	0.19-200	6.41 (n=23)	17.8 (n=10)	2.78	0.357
(Asta Medica AG, Frankfurt/Main, Germany)		2.37-17.6	4.49-18.9		
Glufosfamide	0.5-522	66.8 (n=8)	51.3 (n=6)	0.76	0.697
(Asta Medica AG, Frankfurt/Main, Germany)		17.7-96.9	19.9-69.7		
Treosulfan	0.002-3.6	0.006 (n=23)	0.077 (n=10)	12.6	0.020*
(Medac, Hamburg, Germany)		0.002-1.31	0.012-3.6		
Melphalan	0.12-131	49.4 (n=14)	32.7 (n=5)	0.66	0.459
(Glaxo Wellcome, Parma, Italy)		20.0-58.6	12.6-74.7		
Thiotepa Lederle	0.16-528	30.6 (n=18)	29.1 (n=7)	0.95	0.785
(Riemser, Greifswald, Germany)		11.4-47.8	18.3-42.9		
Prednisolone	0.02- 694	303 (n=28)	304 (n=15)	1.00	0.452
(Jelfa, Jelenia Gora, Poland)		171-375	165-449		
Vincristine	0.02-21	4.33 (n=28)	7.38 (n=15)	1.70	0.236
(Oncovin, Eli-Lilly, Indianapolis, IN, USA)		1.28-8.12	2.80-10.44		
L-asparaginase	0.003-10	1.17 (n=29)	1.21 (n=15)	1.03	0.380
(Medac, Hamburg, Germany)		0.33-8.20	0.50-10.0		

IC_{50}: value of *in vitro* resistance, given in U/L for L-asparaginase and in mM for other drugs; RR: relative resistance = median IC_{50} (relapsed AML) / median IC_{50} (remission AML); n: the number of patients; *p*-value: Mann-Whitney *U*-test; * significant differences between remission and relapse.

etoposide in pediatric AML is related to the use of these drugs which so far have not been tested (treosulfan) or not widely tested (fludarabine). It has shown that these drugs might have a role in the therapy of childhood AML (15), especially treosulfan and fludarabine which have not really been used as cytotoxic agents for AML but more as biochemical modulators for cytarabine (16).

In conclusion, *ex vivo* drug resistance to fludarabine, treosulfan and etoposide is of predictive value in childhood AML undergoing HSCT. Therefore, the drug resistance profile may be used for better stratification of children with AML indicating those patients who may be cured by chemotherapy based on these drugs and to identify those patients who are at high risk of treatment failure and who

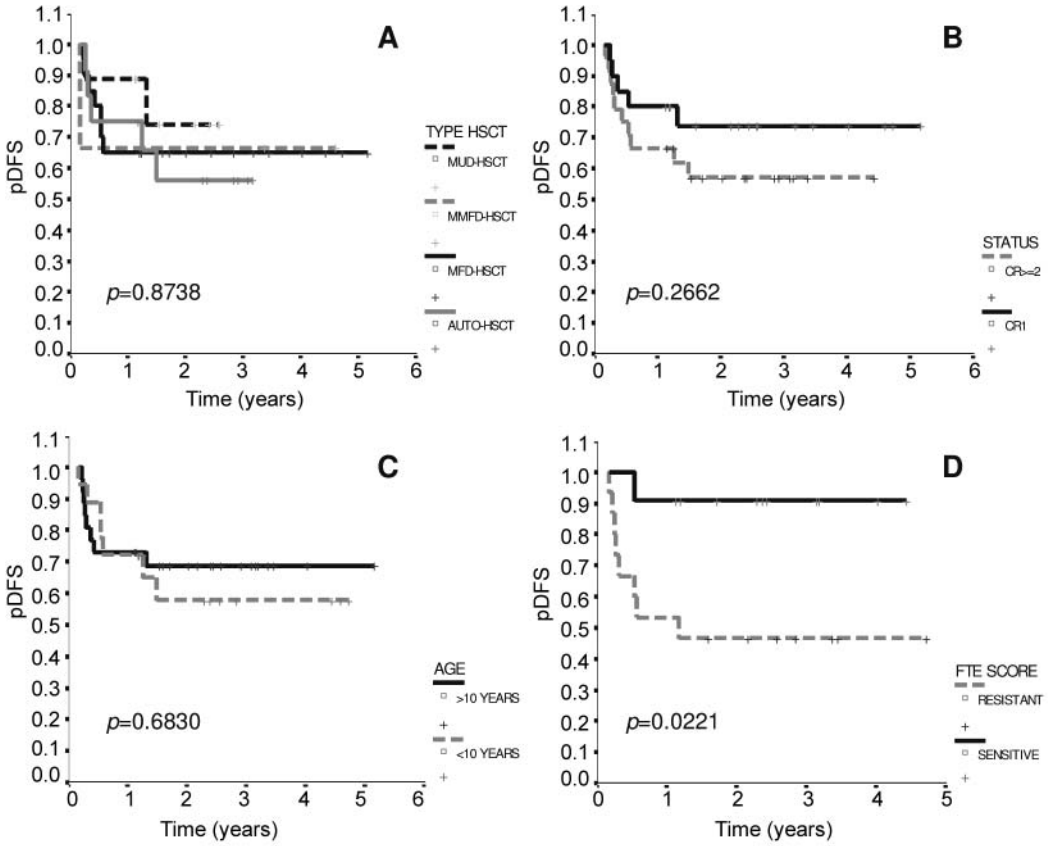

Figure 1. *Impact of (A) type of transplantation (matched unrelated donor, MUD; matched family donor, MFD; mismatched family donor, MMFD; autologous, AUTO), (B) disease status (first complete emission CR1 vs. CR≥2), (C) patient age, (D) combined ex vivo drug resistance to fludarabine, treosulfan, and etoposide (FTE score) on pDFS after HSCT in childhood AML.*

therefore may benefit from more intensive treatment at initial diagnosis, or require additional post-transplant therapeutic strategies.

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